International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2023; 8(4): 224-226

www.veterinarypaper.com
Received: 10-04-2023
Accepted: 02-05-2023
Publish Date: 20-07-2023

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Oxidative stress markers in subclinical and clinical mastitis in Cattles of Bikaner, Rajasthan State

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Abstract
Mastitis is the most common disease affecting large numerous of cattle. The study was carried out by him on 270 seemingly healthy cows in the Bikaner district over a period of seven months. Of these 270 cows tested, 25 control cows and 25 group cows had subclinical mastitis based on the modified California mastitis test. This study was performed to determine biomarkers of oxidative stress (malondialdehyde, reduced glutathione, vitamin E). Oxidative stress studies revealed a significant (p<0.05) increase in malondialdehyde and a decrease in glutathione levels. Plasma vitamin E levels were significantly (p<0.05) lower in clinically affected cows compared to healthy cows. No significant difference was found in vitamin E levels between asymptomatic (subclinical) and control groups.

Keywords: Oxidative stress, reduced glutathione, vitamin E

1. Introduction
Mastitis is recognized as the most important complex and expensive disease affecting the world's dairy industry. It can be described as an inflammation of the parenchyma in the mammary glands and is characterized by physical, chemical, and usually bacterial changes in the milk and pathological changes in the glandular tissues. This production disease is considered an important disease of dairy cows which results in a reduction of milk production, loss in milk quality and quantity, losses due to discarded milk, premature culling, treatment costs, and extra labor cost. It is difficult to detect sub-clinical mastitis (SCM) and its absence results in major losses in milk production. As a result of CM, there are certain clinical manifestations such as swelling of the udder, flakes and clots of milk, and a watery milk.
Physiological stress is associated with rapid differentiation of the secretory parenchyma, intensive mammary gland growth, initiation of milk synthesis and secretion, and is associated with high energy and oxygen requirements (Kohen and Nyska 2002) [9]. An increase in oxygen demand leads to a greater production of reactive oxygen metabolites. As a consequence of oxidative stress, dairy animals may suffer from mastitis and other pathological conditions (Lykkesfeldt and Svendsen, 2007) [10]. Due to the fact that it causes reactive oxygen species and proteolytic enzymes, it results in damage to the secretory epithelium of the mammary gland, which ultimately reduces the amount of milk produced (Celi, 2010) [5]. It is well known that reactive oxygen species (ROS) and nitrogen species (NOS) are generated at a much higher rate than expected during a pathological situation. Consequently, lipid peroxidation occurs as a result of the peroxidation of lipids, causing structural damage to membranes and therefore producing secondary products (Catala, 2006) [4].
In veterinary medicine, oxidative stress has become a major research area. A limited number of conditions in ruminant medicine have been investigated for effects of oxidative stress (Lykkesfeldt and Svendsen, 2007) [10]. It has been known that oxidative stress markers may changes in clinical and subclinical mastitis, these are- reduced glutathione, malondialdehyde, and vitamin E (Ranjan *et al*., 2005; Kizil *et al*., 2007) [11, 8].

2. Materials and Methods
2.1 Animals
In this study, pooled milk samples from 270 apparently healthy cows were tested for subclinical mastitis using the modified California Mastitis Test.

Of these 270 cows tested, 25 CMT-negative controls and 25 CMT-positive cows were evaluated for oxidative stress. Twenty-five cases of clinical mastitis were also evaluated for oxidative stress by physical examination of the udder and milk.

To measure oxidative stress markers (malnoaldehyde and reduced glutathione) from blood, blood samples in EDTA were collected from the jugular vein of each animal (control, clinical, asymptomatic). Biochemical analysis studies were conducted at the National Equine Research Center in Bikaner and the Veterinary Education Complex in Bikaner. Malondialdehyde and reduced glutathione were measured from blood. Plasma was used to measure vitamin E concentrations.

2.2 Collections of milk samples

collect the milk aseptically, every teat became wiped off by means of sprit swab. 2-3 stripping of fore milk had been discarded. Approximately 30ml of fore milk from every teat became amassed in sterilized take a look at tubes.

2.3 California mastitis test

The California Mastitis Test is the most commonly used test for diagnose subclinical mastitis and is convenient for use in the field. MCMT was evaluated in milk samples using Surya *et al*. carried out. (2002) Methods. Briefly, tests were performed using 3-4 ml milk samples collected from each quarter in each of the four cups on the CMT paddle. Add an equal amount of California Mastitis Test Reagent to each paddle cup. A gentle circular motion of the paddle mixed the contents in the horizontal plane and formed a sediment. Depending on the degree of precipitation and gelation, positive test results were classified into negative, trace, weak positive (+), definite positive (++), and strong positive (+++) categories.

3. Result and discussion

3.1 Malondialdehyde

Table 1: Mean± SE values of Malondialdehyde (Nanomole/gm Hb) in control, clinical, subclinical mastitis group

Group	Malondialdehyde (nanomole/gm Hb) (Mean ± SE value)	Range (nanomole/gm Hb)
Control	44.06±2.55[a]	14.6-79.36
Clinical	88.42±8.36[b]	47.00-210.15
Subclinical	61.14±2.94[b]	45.5-111.70

A, b different superscript differ significantly ($p<0.05$)

Malondialdehyde is one of the most commonly indicated marker for lipid peroxidation (Nielsen *et al*., 1997). In this study blood malondialdehyde level in control, clinical mastitis and subclinical mastitis were 44.06±2.55, 88.42±8.36 and 61.14±2.94 (nanomole/gm Hb) with range of 14.6-79.36, 47.00-210.15 and 45.5- 11.70, (nanomole/gm Hb), respectively.

In this study, the increase in malondialdehyde concentration in the clinical group compared to the control group was found to be highly significant ($p<0.001$). Malondialdehyde levels were significantly increased in the asymptomatic mastitis group compared with the control group ($p<0.05$). Elevated MDA plasma concentrations are due to overproduction of reactive oxygen species such as hydroxyl radicals by activated neutrophils from clinically inflamed mammary glands, which can cause peroxidative damage to membranes.

Yang *et al*., 2013 [16] reported that mean malondialdehyde levels were significantly higher in the asymptomatic mastitis group compared to controls. Ghasemian *et al*., 2011 [6] reported elevated plasma malondialdehyde concentrations in cows with subclinical mastitis. Jhambh *et al*., 2013 [7] showed a significant increase in erythrocyte lipid peroxidation in cows with clinical mastitis. Suryasa Saporn *et al*. MDA reported the highest MDA in quarters with clinical mastitis compared with quarters with subclinical mastitis and healthy quarters. Atrosi *et al*. In 1996, we reported that serum lipid peroxidation levels in subclinical mastitis were increased compared to those in healthy cows. The results of the present study were consistent with these findings.

3.2 Reduced glutathione

Table 2: Mean ± SE value of reduced glutathione (mg/gm Hb) in control, clinical and subclinical mastitis group.

Group	Reduced glutathione (mg/gm Hb) Mean ±SE	Range (mg/gm Hb)
Control	2.22±0.096[a]	1.5-3.30
Clinical	3.10± 0.094[b]	2.4- 4.32
Subclinical	3.07± 0.130[b]	2.04-4.27

a, b different significantly ($p< 0.05$)

The mean blood level of reduced glutathione in control, clinical mastitis and subclinical mastitis were 2.22±0.096, 3.10±0.094 and 3.07±0.130 (mg/gm Hb) with range of 1.5-3.30, 2.4-4.32 and 2.04-4.27 (mg/gm Hb), respectively.

The present study revealed a highly significant increase in reduced blood glutathione levels in the clinical and asymptomatic mastitis groups compared to controls ($p<0.01$). Increased glutathione levels may be due to the conversion of reduced to oxidized forms due to overproduction of reactive oxygen species from inflamed glands, which are activated by GSH-dependent enzymes (GSH peroxide and GSH reductase) may be explained. This can lead to intensive regeneration of the oxidized (GSSG) to reduced (GSH) form obtained after reduction of the peroxide to the alcohol. Kizil *et al*., 2007 [8] reported increased plasma GSH concentrations in cows with clinical mastitis. The results of this study were consistent with these findings.

equivalents of glutathione circulate in the blood primarily as cystine, an oxidized and more stable form of cysteine. Cells take up cysteine from the blood, convert it back to cysteine, and use it to synthesize GSH. Glutathione is a very important cytoprotectant. Glutathione's primary function is to reduce oxidative stress. It directly scavenges reactive hydroxyl free radicals, other oxygen-centered free radicals, and radical centers on DNA and other biomolecules. GSH is involved in vitamin C and E recycling, blocks free radical damage, and enhances antioxidant activity (William, 2003).

3.3 Vitamin E

Table 3; Mean ±SE value of vit E (mg/lit) in control, clinical and subclinical mastitis group

Group	Vitamin E (mg/litre) (Mean ± SE)	Range (mg/liter)
Control	4.04± 0.22 [a]	2.47-5.72
Clinical	3.21±0.24[b]	1.20-5.81
Subclinical	3.38±0.25	1.46-5.75

a, b different superscript differ significantly ($p<0.05$)

Plasma vitamin E level in control, clinical mastitis, subclinical mastitis group 4.04±0.22, 3.21±0.24 and 3.38 ±0.25 (mg/litre) with range of 2.47-5.72, 1.20-5.81 and 1.46-5.75 (mg/litre), respectively. The results showed that vitamin E levels were significantly lower in the clinical group compared to the control group. No significant difference was found between the subclinical group and the control group. The reason of decreased concentration of vitamin E could be due to the over utitized of vitamin E to neutralize the excessive reactive oxygen species in inflammatory condition.

Batra *et al*., 1991 [2] reported that plasma and milk levels of vitamin E concentration was low in mastitis milk than healthy cows. The result of this present study was similar to these findings. Kizil *et al*., 2007 [8] reported that vit. E concentration was high in the control group than in the clinical and subclinical mastitis group, but the differences were not statistically significant.

Vitamin E, a potent peroxyl radical scavenger, is a chain breaking antioxidant that prevents the propagation of free radical damage in biological membranes (Traber *et al*., 1995) [14]. Because α- tocopherol can compete for peroxyl radical much faster than can polyunsaturated fatty acids, a small amount of α- tocopherol is able to protect a large amount of polyunsaturated fat (Burton *et al*., 1983) [3].

4. Conclusion

Biochemical studies of oxidative stress parameters reveales significant hight level of malondialdehyde and reduced glutathione in clinical and subclinical group and significant decreased in vitamin E concentration in clinical mastitis group as compare to healthy cows. The high level of malondialdehyde indicated that there was increased erythrocytic lipid peroxidation. Significantly higher levels of reduced glutathione in cows with asymptomatic and clinical mastitis indicated enhanced antioxidant signaling pathways leading to increased peroxide neutralization. A significant drop in the Vitamin E value indicates overuse of Vitamin E to fight free radicals.

5. Acknowledgement

In this study, all funding and facilities were provided by the National Research Center on Equine in Bikaner, as well as the College of Veterinary and Animal Science in Bikaner.

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